

BASIC RESEARCH

Passive stiffness of rat skeletal muscle undernourished during fetal development

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OBJECTIVES: The aim of the study was to investigate the effect of fetal undernutrition on the passive mechanical properties of skeletal muscle of weaned and young adult rats.

INTRODUCTION: A poor nutrition supply during fetal development affects physiological functions of the fetus. From a mechanical point of view, skeletal muscle can be also characterized by its resistance to passive stretch.

METHODS: Male Wistar rats were divided into two groups according to their mother's diet during pregnancy: a control group (mothers fed a 17% protein diet) and an isocaloric low-protein group (mothers fed a 7.8% protein diet). At birth, all mothers received a standardized meal ad libitum. At the age of 25 and 90 days, the soleus muscle and extensor digitorum longus (EDL) muscles were removed in order to test the passive mechanical properties. A first mechanical test consisted of an incremental stepwise extension test using fast velocity stretching (500 mm/s) enabling us to measure, for each extension stepwise, the dynamic stress (σ_d) and the steady stress (σ_s). A second test consisted of a slow velocity stretch in order to calculate normalized stiffness and tangent modulus from the stress-strain relationship.

RESULTS: The results for the mechanical properties showed an important increase in passive stiffness in both the soleus and EDL muscles in weaned rat. In contrast, no modification was observed in young adult rats.

CONCLUSIONS: The increase in passive stiffness in skeletal muscle of weaned rat submitted to intrauterine undernutrition it is most likely due to changes in muscle passive stiffness.

KEYWORDS: Passive stiffness; Skeletal muscle; Fetal; Development; Undernutrition.

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INTRODUCTION

Numerous studies have shown the influence of nutrient supply on development in utero.¹⁻⁴

A poor nutrition supply during fetal development affects physiological functions of the fetus and has long-term consequences at adulthood.^{5,6} This concept of "programming" represents the mechanism whereby a stimulus or an insult during a critical developmental period has permanent effects on structure, physiology, and metabolism.⁷ There is evidence of programming affecting structure and function of skeletal muscles postnatally.⁴ For example, Ozanne et al.^{3,8} showed alterations in muscle metabolic capacities in rats undernourished during fetal development and lacta-

tion. Modifications in muscle fiber type distribution in both young and adult mammals have been reported as well as a decrease in the fiber density in the diaphragm of pups whose mothers had suffered nutritional deprivation.^{1,9,10} However, few studies have examined the consequence of early undernutrition in mechanical muscle properties.

In a previous study, we have shown modifications in both contractile and series elastic properties of rat muscles undernourished during fetal development.¹¹ From a mechanical point of view, skeletal muscle can also be characterized by its resistance to passive stretch. From a functional point of view, muscle passive properties are important to take into account because (1) these characteristics contribute in part to the maximal joint range of motion, (2) part of the force developed by the contracting muscle will be devoted to the stretch of passive antagonist and (3) a relation between passive stiffness and spindle discharge has been shown.¹²

The aim of this study was to evaluate the effect of a low-protein diet during fetal life on the passive mechanical

properties of a postural muscle (soleus) and a nonpostural muscle (extensor digitorum longus, EDL). This study was conducted in weaned rats and in young adult rats to analyze the short- and long-term effects of this early nutritional manipulation.

MATERIALS AND METHODS

Experimental animals

Virgin female Wistar rats (body mass 281.86 ± 14.97 g) were housed individually with males under standardized conditions. On the day the copulation plug was found, the females were isolated and assigned to one of two experimental groups: a control group (C, $n=11$) and an undernourished group (UN, $n=11$). During gestation, rats of group C were fed a control diet (17% protein) according to the recommendations of AIN-93G,¹³ and UN animals received a low-protein isocaloric diet (7.8%) *ad libitum* (Table 1). On the first day after birth, all mothers received a control diet (17% of protein) *ad libitum* and litters were limited to six male pups per mother. At weaning, pups were fed a standardized meal (17% of protein) *ad libitum* until 60 days old.¹³ Afterwards, offspring received a 12% protein diet *ad libitum* until 90 days of age.

The protocols used in the present study were in accordance with the guidelines and regulations of the Ethical Hygiene and Safety Committee of the Compiègne University of Technology.

Biomechanical analysis

Rats 25 days old ($n=14$) and 90 days old ($n=16$) were anesthetized with an intraperitoneal injection of sodium pentobarbital (30 mg/kg of body mass). The soleus and EDL muscles were carefully excised from the hind limb and placed in a dissection chamber containing Ringer's solution (composition in mM: NaCl, 115; NaHCO₃, 28; CaCl₂, 2.5, MgSO₄, 3.1, KCl, 3.5, KH₂PO₄, 1.4; glucose, 11.1) maintained at 25°C and oxygenated with a gas mixture of 95% O₂ and 5% CO₂ that resulted in a pH of 7.3. At the end of the experiment all animals was killed in accordance with the animal committee at Compiègne University of Technology. The proximal part of the muscle was fixed to a force transducer and the distal extremity was linked to the

moving part of a servocontrolled ergometer described in detail elsewhere.¹⁴ The muscle was adjusted from its slack length (L_s), i.e., the muscle length from which a resting tension of 10 mN was obtained. It was then submitted to two different procedures: an incremental stepwise extension test and a stretch-release test at slow velocity. For each test type, three tests were performed, the first two tests were used for preconditioning the muscle and the third test served for data analysis.

With regard to the incremental stepwise extension test, the muscle was stretched by four successive stepwise extensions, initially imposed from L_s . Each stepwise extension consisted of a 5% L_s step at fast velocity (500 mm/s) that was maintained for 80 s to observe a reduction in tension toward a plateau value. After the fourth stepwise extension, the muscle was suddenly released to L_s . This test enabled us to measure, for each extension stepwise, the dynamic force (F_d) that corresponded to the maximal force developed by muscle at the end of the fast extension and the steady force (F_s) at the end of the plateau in length (Fig. 1). Then, F_d and F_s were divided by the physiological cross-sectional area (PCSA) of the muscle, which yields the dynamic tension (σ_d) and the steady tension (σ_s). PCSA of muscle was calculated using the equation $PCSA = MW / (1.06 \times L_f)$, where MW is muscle mass, 1.06 is the muscle density (in g/cm³), and L_f is the fiber length. L_f corresponds to 72% and 44% of the length of the soleus and the EDL muscles, respectively.^{15,16}

The stretch-release test consisted of stretching the muscles at amplitude up to 125% L_s with a slow velocity (0.1 mm/s) following by a release until L_s with the same velocity (Fig. 2). From these data, stress (i.e., passive force normalized in respect of PCSA) and strain (i.e., deformation/ L_s) were calculated to construct the stress-strain curve. From this curve, tension at 125% L_s ($F_{125}/PCSA$), stiffness at 125% L_s and tangent modulus (i.e., slope in the linear portion of the stress-strain curve) were calculated.

Statistical analysis

All data are presented as mean \pm SEM. A two-way (to evaluate the effect of the age and diet on body mass) and three-way (to evaluate the effect of age, diet and muscle on the other parameters) Analysis of variance (ANOVA) for repeated measurements followed by the Holm Sidak post hoc test were performed. A level of 95% was set as the statistical difference. The statistical treatment of the data was performed with the Sigmasat software (Systat Software, Inc., Chicago, IL).

RESULTS

The body mass of pups from UN mothers was significantly lower at 25 days (92.6 ± 5.18 g vs 71.3 ± 1.3 g for the C and UN groups, respectively; $p<0.05$) and 90 days (449.5 ± 9.2 g vs 413.2 ± 14 g for C and UN group, respectively; $p<0.05$).

Absolute and relative mass of the soleus and EDL muscles was significantly smaller in the UN group than in C group in weaned and young adult rats (Fig. 3).

Results of the incremental stepwise extension test indicated increases in resistance to passive stretch for each extension in both soleus and EDL muscles in weaned rats (Fig. 4). At this age, soleus muscle of UN rats showed an increase in dynamic tensions by 40%, 48%, 57%, and 52% for

Table 1 - Composition of the experimental diets.

Ingredient (g/kg)	Control diet (17% protein)	Low-protein diet (7.8% protein)
Casein (≥ 92.5 protein)	183.8	84.3
Cornstarch	645.7	745.2
Soybean oil	70.0	70.0
Fibre	50.0	50.0
Vitamin mix*	10.0	10.0
Mineral mix†	35.0	35.0
Choline bitartrate	2.5	2.5
L-methionine	3.0	3.0
Total	1000	1000

*American Institute of Nutrition 93G;¹³ vitamin mix provided (mg/kg diet): nicotinic acid 30.0, calcium pantothenate 16.0, pyridoxine-HCl 7.0, thiamin-HCl 6.0, riboflavin 6.0, folic acid 2.0, biotin 2.0, cyanocobalamin, 25.0, α -tocopherol 150.0, retinyl palmitate 8.0, cholecalciferol 2.5, and phyloquinone 0.75.

†American Institute of Nutrition 93;¹³ mineral mix provided (mg/kg diet): calcium 5.0, phosphorus 1.6, sodium 1.0, potassium 2.3, magnesium 0.5, iron 0.03, zinc 0.03, and copper 0.01.

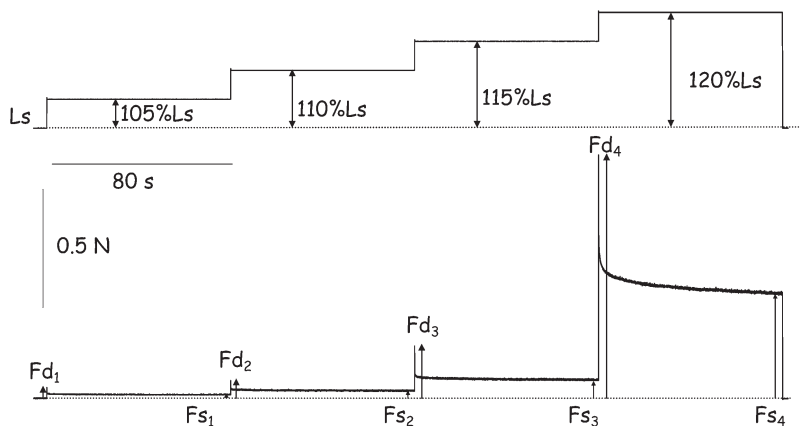


Figure 1 - Typical recording of passive tension induced by an incremental stepwise extension test. F_d : dynamic force; F_s : steady force. Upper trace, change in length; bottom trace, change in force.

the first, second, third, and fourth increment, respectively (Fig. 4). Similar increases were obtained in EDL muscle. In addition, undernutrition induced an increase in steady tension of about 65% in the soleus and 100% in the EDL (Fig. 4). At 90 days, no difference in either dynamic tension or steady tension was observed in the soleus and the EDL between the control group and the undernourished group.

Passive force developed at 25% strain during the stretch-release test and was not modified in the soleus and EDL muscles of weaned and young adult rat (Fig. 5). When passive force was expressed in terms of normalized tension (i.e., force divided by PCSA), there was an increase in the passive tension in both the soleus and the EDL muscles in weaned rats (Fig. 5). This increase in resistance to passive stretch observed in the soleus and EDL of the UN group was also confirmed by the increase in the tangent modulus and in normalized stiffness. In young adult rats, no difference was observed in these parameters between groups in either the soleus or the EDL muscles.

DISCUSSION

The results of present study are in accordance with numerous studies showing poor maternal nutrition during gestation affects fetal growth and development.^{1,3,6,8,11}

Thus, the decrease in the pup weight can be associated with the availability of nutrients for transfer to the fetus, possibly involving metabolic parameters such as glucose and insulin.¹⁷ Consequent to maternal nutrient restriction, the soleus and EDL muscle weight was significantly reduced in weaned and young adult rats. This muscle atrophy is consistent with the programming of skeletal muscle insulin sensitivity during fetal development⁸ as it has been shown that insulin-sensitive tissues undergo important changes in response to maternal protein restriction.^{18,19}

A previous study had shown that maternal protein restriction during gestation induced changes in both contractile and series elastics properties.¹¹ In addition to these mechanical changes, the present work has demonstrated that the passive elastic properties are also changed by this early nutritional manipulation. In effect, even if passive force that developed during the slow velocity stretch was not different between nutritional groups, the normalized tension showed an increase in soleus and EDL muscles in UN weaned rats. This increase in resistance to passive stretch was also perceived by the increase in the normalized stiffness and the increase in the tangent modulus. Results of the incremental stepwise test showed that passive stiffness was increased during both the

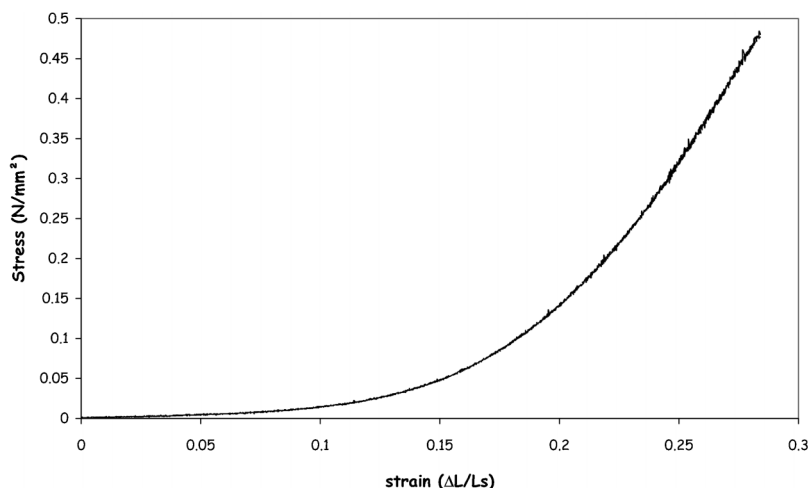


Figure 2 - Typical stress-strain curve obtained from the stretch-release test.

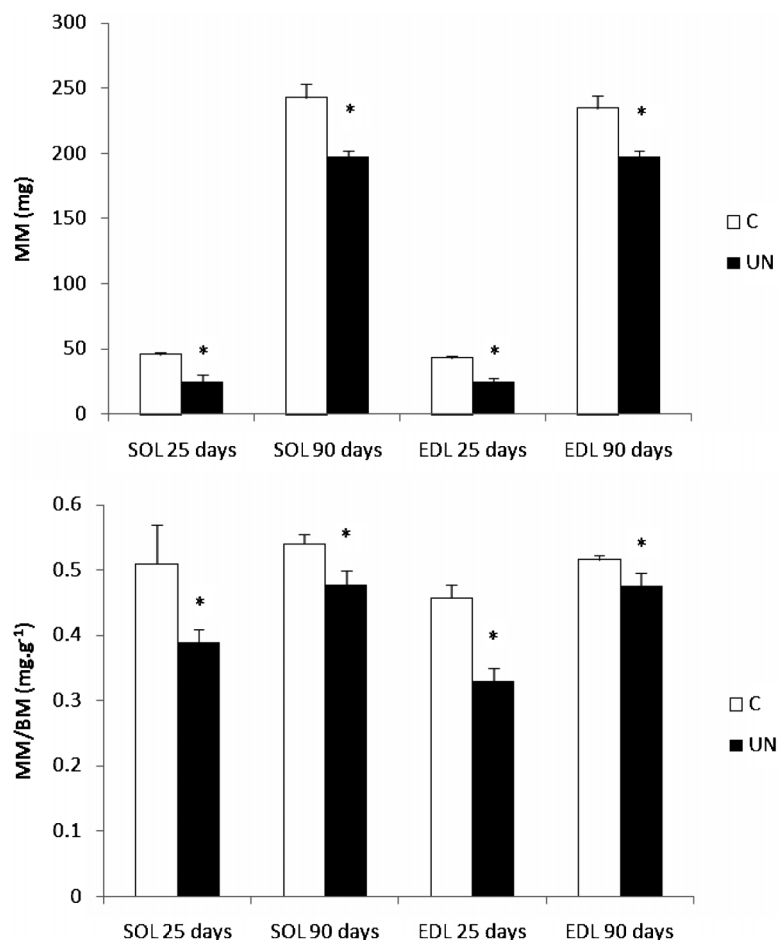


Figure 3 - Absolute muscle mass (MM) and ratio muscle mass to body mass (MM/BM) of soleus and EDL muscles at 25 and 90 days of age. C: control group; UN: undernourished group. *p<0.05 vs control.

dynamic and the static phases and for short and long stretches.

Muscle passive stiffness is a function of the parallel elastic component described in Hill's model.²⁰ Its properties are affected by membrane structure and specifically by the concentration and type of collagen.²¹⁻²⁵ The effects of nutritional status on the regulation of skeletal muscle collagen content are varied. Roy et al.²⁶ reported no influence of nutritional level in muscle collagen content in pectoralis muscle of broilers but with some differences in the collagen structure of the perimysium. In the gastrocnemius muscle of adult mice deprived of food for 2 days, Jagoe et al.²⁷ showed a decrease in gene expression for many extracellular matrix proteins like collagen. More recently, Stevenson et al.²⁸ studied the transcriptional profile of myotube under starvation conditions. These authors reported a downregulation of genes involved in collagen synthesis and maturation. Nevertheless, the effect of nutritional supply during fetal development seems to be different in the development of connective tissue in skeletal muscle. In swine, Karunaratne et al.²⁹ reported that the smallest littermate, reflecting a poor level of in utero nutritional supply, contained a higher concentration of type I collagen than the largest littermate. In our study, such an increase in the content of collagen could explain the increase in the passive tension observed in the soleus and EDL muscles of undernourished rats.

In addition to collagen, other connective proteins are a source of muscle passive tension. Titin, a 3-MDa elastic filamentous protein, links the Z line to the myosin filament in sarcomeres. Wang et al.³⁰ reported that passive elastic properties of muscle fibers are related to expression of the titin isoform. Moreover, Toursel et al.³¹ showed a decrease in passive tension in soleus fiber of unloaded rat in relation to a decrease in titin content. Passive stiffness results also from the relation between endosarcomeric and exosarcomeric protein networks constituted by different structural proteins like desmin.³² Lastly, telethonin (Titin-cap), an important component of the N-terminal titin anchor in the Z line,³³ seems to act on passive stiffness.³⁴ Interestingly, it has been shown that nutritional status changes the gene expression of these proteins. Byrne et al.³⁵ reported an upregulation of cytoskeletal proteins like desmin or telethonin in the muscles of steers after nutritional restriction. Oumi et al.³⁶ reported muscle ultrastructure damages induced in rats nourished with a low-protein diet for 2 weeks after weaning. More precisely, they showed disorganization in some sarcomeres, with a disruption of the Z line appearing jagged. As postulated by Oumi et al.,³⁶ these sarcomere damages could be the result of the "disintegration" of structural proteins like desmin and titin. Muscle disorganization, such as those observed by these authors, could induce an increase in passive stiffness. As a matter of fact, Anderson et al.³⁷ reported an increase in

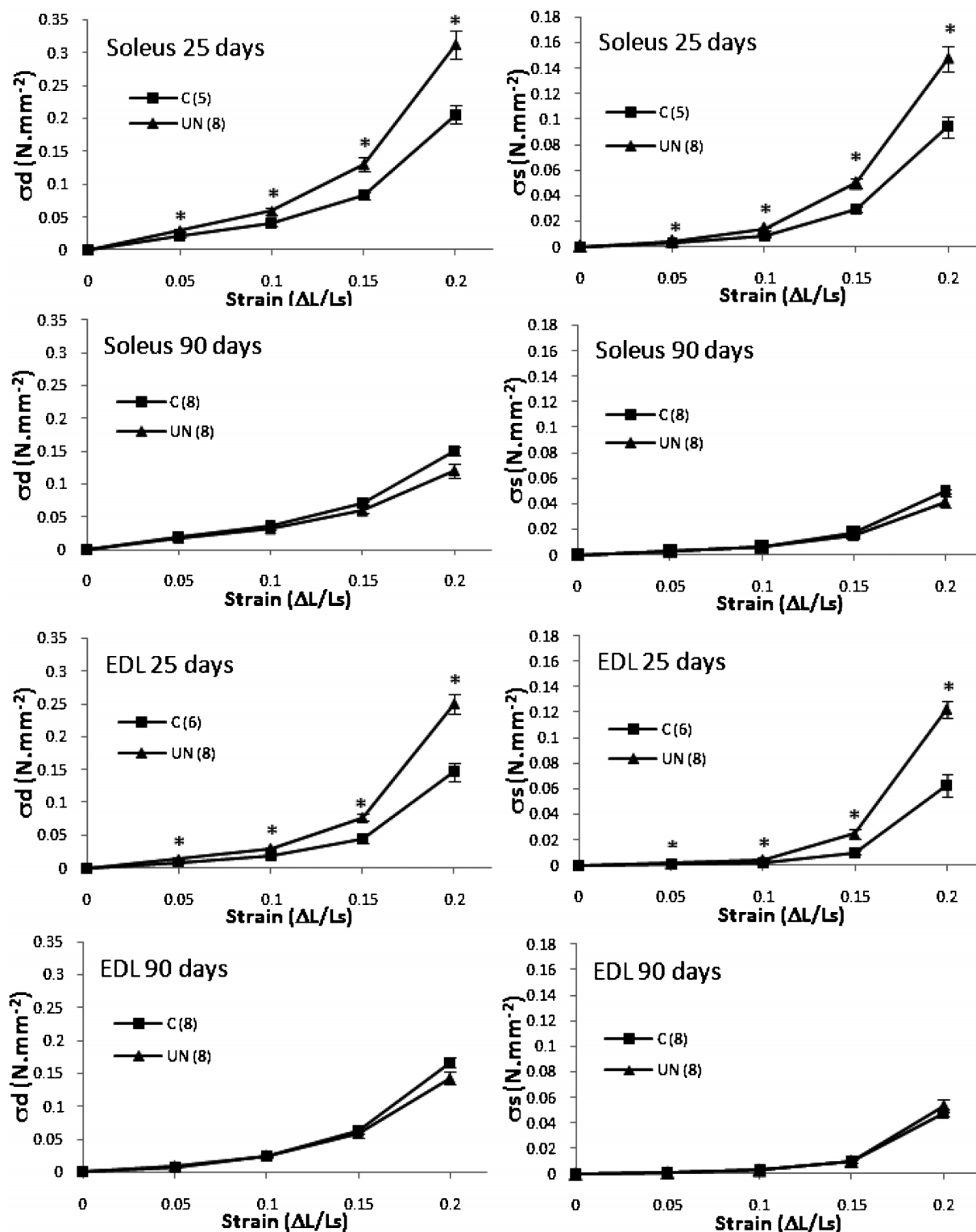


Figure 4 - Effects of undernutrition on dynamic tension (σ_d) (left) and steady tension (σ_s) (right) in soleus and EDL muscles at 25 and 90 days of age induced by incremental stepwise extensions. C: control group; UN: undernourished group. The number of muscles is indicated in parentheses. * $p < 0.05$ vs control.

passive stiffness in desmin knockout mice and ascribed this mechanical change to the adaptation of passives structures consequent to the lack of desmin.

Lastly, no modification in the passive stiffness properties was observed between groups in young adult rats. The total

recovery of these elastic properties reveals that the changes observed in the weaning rats can be completely reversed after nutritional recovery before the animal reaches the adult age. Nevertheless, it will be interesting to evaluate older animals in order to confirm or invalidate that the in

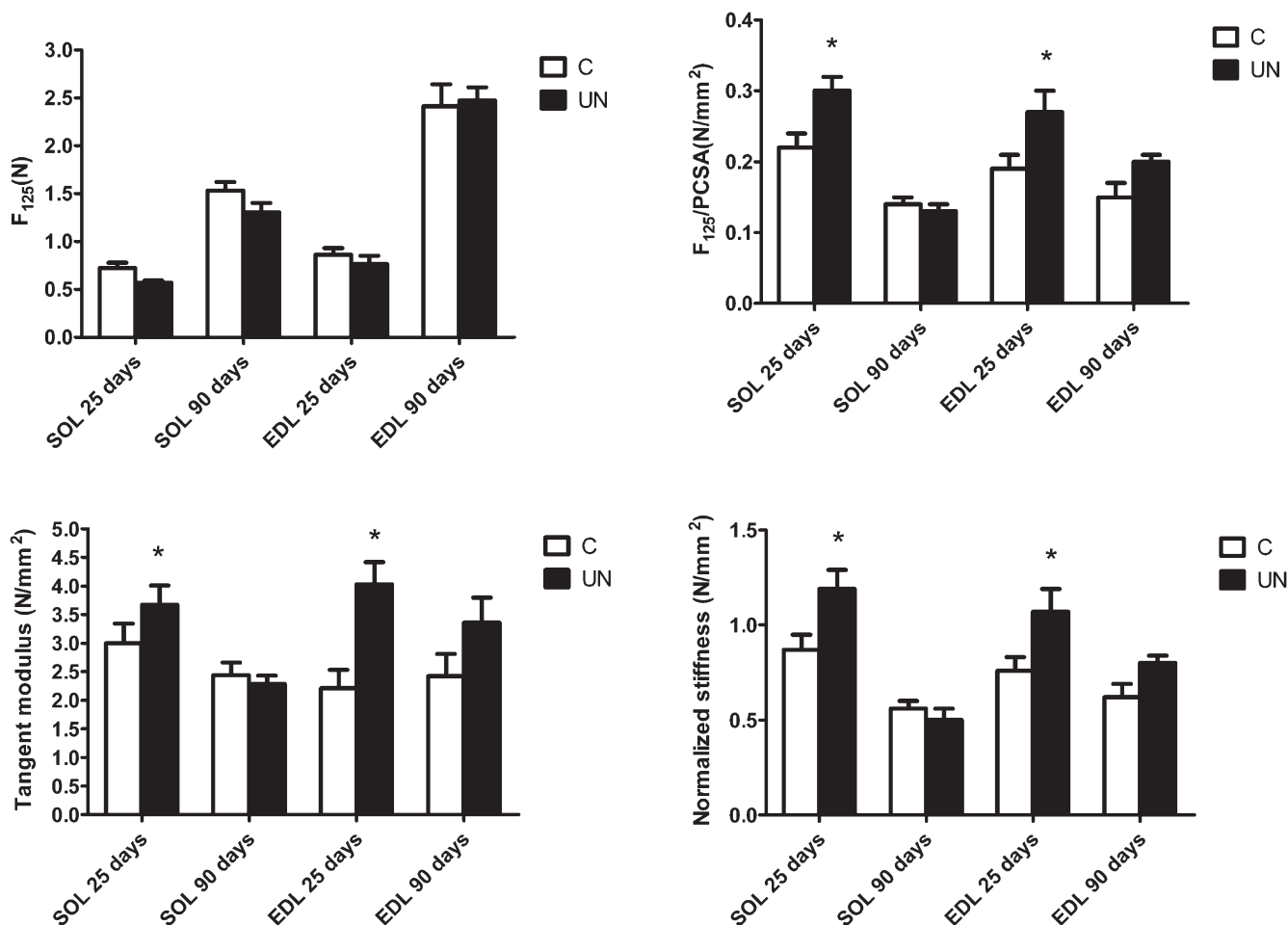


Figure 5 - Effects of undernutrition on tension at 125% L_s (F_{125}), $F_{125}/PCSA$, stiffness at 125% L_s and tangent modulus in soleus and EDL muscles at 25 and 90 days of age induced by the stretch-release test. L_s : slack length; $PCSA$: physiological muscle cross-sectional area; C: control group; UN, undernourished group. * $p < 0.05$ vs control.

utero low-protein diet supply has no long-term consequences in muscle passive mechanical properties.

CONCLUSIONS

This study has permitted understanding of the effect of a prenatal undernutrition on the passive elastic component of the postural muscle (soleus) and a nonpostural muscle (EDL). Prenatal undernutrition showed short-term alterations in passive stiffness that can be explained in terms of adaptations in passive structures and/or distribution of endosarcomeric and exosarcomeric proteins in the skeletal muscle. However, further biochemical investigations are necessary to establish the effects of this particular nutritional manipulation in a noncontractile protein profile of skeletal muscle.

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